Application No.: U.S. National Stage of PCT/EP2003/008199

DT01 Rec'd PCT/CTC 2 0 JAN 2005

AMENDMENTS TO THE CLAIMS

- 1. (Original) A process for the oxidation of carotenoids, which comprises reacting a carotenoid in the presence of an enzyme having cytochrome P450 monooxygenase activity from bacteria of the genus *Thermus sp.*, and isolating the oxidation product.
- 2. (Original) A process as claimed in claim 1, wherein
 - al) a recombinant microorganism which produces an enzyme having cytochrome P450 monooxygenase activity is cultivated in a culture medium in the presence of exogenous β-carotene or β-carotene formed as intermediate; or
 - a2) a β-carotene-containing reaction medium is incubated with an enzyme having cytochrome P450 monooxygenase activity; and
 - b) the oxidation product formed or a secondary product thereof is isolated from the medium.
- 3. (Original) A process as claimed in claim 2, wherein the oxidation product comprises zeaxanthin, cryptoxanthin, adonirubin, astaxanthin, lutein or mixtures thereof.
- 4. (Currently amended) A process as claimed in claim 2, in any of the preceding claims, wherein the oxidation is carried out by cultivating the microorganism in the presence of oxygen at a cultivation temperature of at least about 20°C and at a pH of about 6 to 9.
- 5. (Original) A process as claimed in claim 4, wherein the microorganism is able, through heterologous complementation, to produce carotenoids and additionally expresses an enzyme having cytochrome P450 monooxygenase activity.
- 6. (Currently amended) A process as claimed in <u>claim 1</u>, any of claims 1 to 3, wherein a <u>the carotenoid is added as exogenous substrate to a medium, and the oxidation is</u>

Application No.: U.S. National Stage of PCT/EP2003/008199 Docket No.: 13111-00001-US

carried out by enzymatic reaction of the substrate-containing medium in the presence of oxygen at a temperature of at least about 20°C and at a pH of about 6 to 9, it being possible for the substrate-containing medium additionally to comprise an approximately 10- to 100-fold molar excess, based on the substrate, of reducing equivalents.

- 7. (Currently amended) A process as claimed in claim 1, any of the preceding claims, wherein the cytochrome P450 monooxygenase has an amino acid sequence which comprises a part-sequence from amino acid residue Pro328 to Glu345 as shown in SEQ ID NO:2.
- 8. (Currently amended) A process as claimed in claim 1, any of the preceding claims, wherein the cytochrome P450 monooxygenase has an amino acid sequence which additionally comprises a part-sequence from amino acid residue Val216 to Ala227 as shown in SEQ ID NO:2.
- 9. (Currently amended) A process as claimed in <u>claim 1</u>, any of the preceding claims, wherein the <u>cytochrome P450</u> monooxygenase has an amino acid sequence which comprises at least one other part-sequence which is selected from part-sequences of at least 10 consecutive amino acids from the sequence regions specified by the amino acid residues Met1 to Phe327 and Gly346 to Ala389 as shown in SEQ ID NO:2.
- 10. (Currently amended) A process as claimed in claim 1, any of the preceding claims, wherein the cytochrome P450 monooxygenase has an amino acid sequence which essentially corresponds to SEQ ID NO:2.
- 11. (Currently amended) A process as claimed in claim 1, wherein a-the cytochrome P450 monooxygenase is from a bacterium of the species *Thermus thermophilus*-is used.

Application No.: U.S. National Stage of PCT/EP2003/008199 Docket No.: 13111-00001-US

12. (Currently amended) A process as claimed in <u>claim 2</u>, <u>any of the preceding claims</u>, where a recombinant microorganism which harbors an expression construct which comprises, under the control of regulatory nucleotide sequences, the coding sequence for a cytochrome P450 monooxygenase as <u>defined in any of claims 7 to 11</u> is cultivated, wherein the cytochrome P450 monooxygenase has an amino acid sequence

- (a) which comprises a part-sequence from amino acid residue Pro 328 to Glu345 as shown in SEQ ID NO:2; and/or
- (b) which comprises a part-sequence from amino acid residue Val216 to Ala227 as shown in SEQ ID NO:2; and/or
- which comprises at least one part-sequence which is selected from partsequences of at least 10 consecutive amino acids from the sequence regions
 specified by the amino acid residues Met1 to Phe327 and Gly345 to Ala389
 as shown in SEQ ID NO:2; and/or
- (d) which essentially corresponds to SEQ ID NO:2; and/or
- (e) which is from a bacterium of the species Thermus thermophilus.
- 13. (Canceled)
- 14. (Original) A recombinant microorganism which is able through heterologous complementation to produce β-carotene and additionally expresses an enzyme having cytochrome P450 monooxygenase activity.
- 15. (Original) A microorganism as claimed in claim 14, in which the heterologous complementation is with carotenogenic genes.

- 16. (Currently amended) A microorganism as claimed in claim 14, either of claims 14 and 15, derived from bacteria of the genus *Escherichia sp*.
- 17. (Currently amended) A microorganism as claimed in claim 16, derived from E. coli, in particular E. coli MJ-109.
- 18. (Currently amended) A microorganism as claimed in claim 14, any of claims 14 to 17, transformed with an expression vector which comprises, under the genetic control of regulatory nucleotide sequences, the coding sequence for a cytochrome P450 monooxygenase as defined in any of claims 7 to 11.
- 19. (Currently amended) An expression vector comprising the coding sequence for a cytochrome P450 monooxygenase as defined in any of claims 7 to 11, which is operatively linked upstream to the strong tac promoter and downstream to the strong rm B ribosomal terminator, wherein the cytochrome P450 monooxygenase has an amino acid sequence
 - (a) which comprises a part-sequence from amino acid residue Pro 328 to Glu345 as shown in SEQ ID NO:2; and/or
 - (b) which comprises a part-sequence from amino acid residue Val216 to Ala227 as shown in SEQ ID NO:2; and/or
 - which comprises at least one part-sequence which is selected from partsequences of at least 10 consecutive amino acids from the sequence regions
 specified by the amino acid residues Met1 to Phe327 and Gly345 to Ala389
 as shown in SEQ ID NO:2; and/or
 - (d) which essentially corresponds to SEQ ID NO:2; and/or

0

(e) which is from a bacterium of the species Thermus thermophilus.

Application No.: U.S. National Stage of PCT/EP2003/008199 Docket No.: 13111-00001-US

20. (New) A process of claim 6, wherein the substrate-containing medium further comprises about 10- to 100-fold molar excess, based on the substrate, of reducing equivalents.

21. (New) A microorganism as claimed in claim 17 derived from E. coli MJ109.